

**WHAT IS CLAIMED IS:**

1. A method of determining the susceptibility of a biofilm to an antimicrobial agent, comprising:
  - culturing microbes on a support to form a biofilm;
  - contacting the biofilm with a metabolic substrate;
  - determining a base-line metabolic activity of the biofilm by measuring a signal from the metabolic substrate;
  - contacting the biofilm with one or more antimicrobial agents;
  - determining an experimental metabolic activity by measuring a signal from the metabolic substrate; and
  - comparing the base-line metabolic activity with the experimental metabolic activity, wherein a change is indicative of an antimicrobial agent that affects microbes in the biofilm.
2. The method of claim 1, wherein the metabolic substrate comprises a fluorogenic or chromogenic moiety.
3. The method of claim 1, wherein the metabolic substrate comprises a member selected from the group consisting of nitroblue tetrazolium chloride BT; 2H-(Tetrazolium,-3,3'-(3,3'-dimethoxy(1,1'-biphenyl)-4,4'-diyl)bis(4-nitro phenyl)-5-(phenyl), dichloride); 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; thiazolyl blue); 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-- phenyltetrazolium chloride (INT); 3-(4-Iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride; neotetrazolium chloride (NTC; 2,2',5,5'-Tetr phenyl-3,3'-[p-diphenylene] ditetrazolium chloride); tetranitro tetrazolium blue chloride (TNBT; 2,2',5,5'-Tetra(4-nitrophenyl)-3,3'-dimethoxy-4,4'-biphenylene)-2H,2H'-dit etrazolium chloride); tetrazolium Blue chloride (BT; blue tetrazolium chloride; 2,2',5,5'-

Tetraphenyl-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-2H,2H'-ditetr azonium chloride); triphenyltetrazolium chloride (TTC; tetrazolium red; 2,3,5-Triphenyl-2H-tetrazolium chloride); triphenyltetrazolium bromide (TTB; 2,3,5-Triphenyl-2H-tetrazolium bromide); 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST 1); 4-[3-(4-Iodophenyl)-2-(2,4-dinitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate (WST 3); 2-Benzothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcarbamoyl)phenyl]-2H-tetrazolium salt (WST 4); 2,2'-dibenzothiazolyl-5,5'-bis(4-di(2-sulfoethyl)carbamoylphenyl)-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)ditetrazolium, disodium salt (WST-5); sodium 3'-{1-[(phenylamino)-carbonyl]-3,2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT); 2-(2'-benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium (BSPT); 2-benzothiazolyl-(2)-3,5-diphenyl tetrazolium (BTDP); 2,3-di(4-nitrophenyl)tetrazolium (DNP); 2,5-diphenyl-3-(4-styrylphenyl) tetrazolium (DPSP); distyryl nitroblue tetrazolium (DS-NBT); 2-phenyl-3-(4-carboxyphenyl)-5-methyl tetrazolium (PCPM); thiocarbamyl nitroblue tetrazolium (TCNBT; 2,2'-Di(p-nitrophenyl)-5,5'-di(p-thiocarbamylphenyl)-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)ditetrazolium chloride); 5-cyano-2,3-di-4-tolyl-tetrazolium chloride (CTC); Nitrotetrazolium Violet (NTV); p-Anisyl Blue Tetrazolium Chloride (pABT); m-Nitro Neotetrazolium Chloride (m-NNT); o-Tolyl Tetrazolium Red (o-TTR); p-Tolyl Tetrazolium Red (pTTR); Piperonyl Tetrazolium Blue (PTB); p-Anisyl-p-Nitro Blue Tetrazolium Chloride (pApNBT); Veratryl Tetrazolium Blue (VTB); and tetrazolium violet (TV; 2,5-Diphenyl-3-(alpha-naphthyl)tetrazolium chloride).

4. The method of claim 1, wherein the support is a Calgary Biofilm Device.

5. The method of claim 1, wherein the support comprises discs in a culture well.

6. The method of claim 5, wherein the discs comprise an acetate material.

7. The method of claim 1, further comprising one or more additional metabolic substrates.

8. An assay device comprising:

a cell culture device comprising a plurality of wells, each well comprising a substantially planar bottom and at least one wall;

a plurality of supports, each support disposed within a well perpendicular to the substantially planar bottom, wherein the plurality of supports comprise discs; and

at least one cover that fittably seals the top of each well.

9. The assay device of claim 8, wherein the cell culture device comprises a 96-well tissue culture plate.

10. The assay device of claim 8, wherein the plurality of supports are acetate discs.

11. The assay device of claim 8, wherein each of the plurality of discs is treated with ethanol and washed prior to being disposed within each well.

12. The assay device of claim 8, wherein the diameter of the discs is equal to, or slightly larger than the diameter of each well.

13. An assay system comprising:

a cell culture device comprising a plurality of wells and/or channels, each well or channel comprising a substantially planar bottom;

a plurality of supports, each support disposed within a well or channel perpendicular to the substantially planar bottom; and

at least one cover that fittably seals the top of each well or channel;

culturing a sample comprising a microbial population in a media within the wells or channels such that the media is in contact with the supports thereby forming a biofilm on the supports;

measuring a fluorometric or colorimetric absorbance from a fluorogenic or chromogenic moiety in the sample;

comparing the fluorogenic or colorimetric absorbance to a standard sample.

14. The assay system of claim 13, wherein the cell culture device comprises a 96-well tissue culture plate.

15. The assay system of claim 13, wherein the plurality of supports comprise discs.

16. The assay system of claim 15, wherein the plurality of supports are acetate discs.

17. The assay system of claim 16, wherein each of the plurality of acetate discs is treated with ethanol and washed prior to being disposed within each well.

18. The assay system of claim 15, wherein the diameter of the discs is equal to, or slightly larger than the diameter of each well.

19. The assay system of claim 13, wherein the sample is an environmental sample.
20. The assay system of claim 13, wherein the sample is obtained from a patient.
21. The assay system of claim 13, wherein the microbial population is substantially homogeneous.
22. The assay system of claim 13, wherein the microbial population comprises a mixed species biofilm.
23. The assay system of claim 22, wherein the mixed species biofilm comprises a population of prokaryotes and eukaryotes.
24. The assay system of claim 23, wherein the eukaryotes comprise fungi or yeast microbes.
25. The assay system of claim 13, wherein the fluorogenic or chromogenic substrate is 2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT).
26. The assay system of claim 13, wherein the support is affixed to the at least one cover.
27. The assay system of claim 25, further comprising:
  - means for measuring a base-line metabolic activity of the biofilm;
  - means for contacting the biofilm with one or more antimicrobial agents;
  - means for measuring an experimental metabolic activity; and

means for comparing the base-line metabolic activity with the experimental metabolic activity, wherein a change is indicative of an antimicrobial agent that affects microbes in the biofilm.